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Arnold J.J. Reuser · Hanneriekc Van den Hout
Agnes G.A. Bijvoet · Marian A. Kroos
Martin P. Verbeet · Ans T. Van der Ploeg

Enzyme therapy for Pompe disease: from science to industrial enterprise

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Abstract Pompe disease or glycogen storage disease type II (OMIM 232300) is a metabolic myopathy with a broad clinical spectrum. Generalised muscle weakness combined with cardiomegaly presents within the first 3 months after birth, if the lysosomal α-glucosidase (AGLU) deficiency is complete. Residual enzyme activity prevents cardiac involvement and delays onset of muscle weakness. Enzyme therapy, by intravenous administration of acid AGLU, aims to supplement the missing enzyme activity. At the SHS symposium on Glycogen Storage Diseases Type I and II, in Fulda, two interim accounts were given of studies on the efficacy of enzyme therapy for Pompe disease; one with recombinant human acid AGLU produced in Chinese hamster ovary cells and the other with the same enzyme produced in the milk of transgenic rabbits. *Conclusion:* this review focuses on the latter study, discusses the scientific, technological and commercial aspects of the enterprise, and addresses the prospects and challenges of enzyme therapy for Pompe disease.

Keywords Acid maltase deficiency · Enzyme therapy · Glycogenosis · Lysosomal storage disease · Transgene technology

Abbreviations *AGLU* α-glucosidase · *CHO* Chinese hamster ovary · *CRIM* cross-reactive immunological material · *M6P* mannose-6-phosphate

A.J.J. Reuser (✉) · A.G.A. Bijvoet · M.A. Kroos
Department of Clinical Genetics,
Erasmus University Rotterdam, PO Box 1738,
3000 DR Rotterdam, The Netherlands
E-mail: reuser@ikg.fgg.eur.nl
Tel.: +31-10-4087153
Fax: +31-10-4089489

H. Van den Hout · A.T. Van der Ploeg
Department of Paediatrics, Division of Metabolic Diseases,
Sophia Children's Hospital, Rotterdam, The Netherlands

M.P. Verbeet
Institute of Chemistry, Leiden University,
Leiden, The Netherlands

Enzyme therapy in historic perspective

Over the past 35 years we have learned what it takes to bring enzyme therapy for lysosomal storage disorders into practice. The concept of enzyme therapy is built on the key function of lysosomes in cell and tissue renewal. Macromolecular compounds, even whole cell organelles like mitochondria, are recycled by the lysosomal system (Fig. 1). Materials derived from the intra-cellular space are sequestered by membranes and delivered through fusion of newly formed autophagic vacuoles with lysosomes. Extra-cellular materials are taken up by bulk or receptor mediated endocytosis and are delivered by fusion of endosomes with lysosomes. Once inside lysosomes, the material is degraded by one or a combination of several lysosomal hydrolases. Some of these hydrolases are assisted by activator proteins [23].

The role of lysosomes in cellular pathology became evident in 1963 with the discovery of acid α-glucosidase (AGLU) deficiency as primary defect in Pompe disease or glycogen storage disease type II [13]. Knowing the function of lysosomes, it was envisaged that patients with lysosomal storage disorders could be treated by administration of the missing enzyme that would find its way to the lysosomes via endocytosis (Fig. 1) [9]. Expectations were high and regulations concerning the performance of clinical studies were less strict in those days than they are at present. The first attempt at enzyme therapy dates from 1964 and involved treatment of a patient with Pompe disease with acid AGLU from the fungus *Aspergillus niger* [5]. Similar experiments followed using enzyme preparations from various other sources [30]. A slight increase of acid AGLU activity in liver was obtained in some of these studies upon intravenous infusion [19], but not in muscle. Reduction of liver lysosomal glycogen only was obtained with high enzyme doses over long periods of time. In all instances, lack of ultimate effect and occurrence of serious side-effects terminated the treatment.

In the period 1965–1980, numerous reports were published about enzyme replacement therapy in several of the lysosomal storage diseases, but most results were basically negative [30]. Some important facts became evident. Enzyme preparations from non-human sources are antigenic. The blood brain barrier cannot be crossed, so that patients with central nervous system involvement cannot be treated for mental and motor dysfunction.

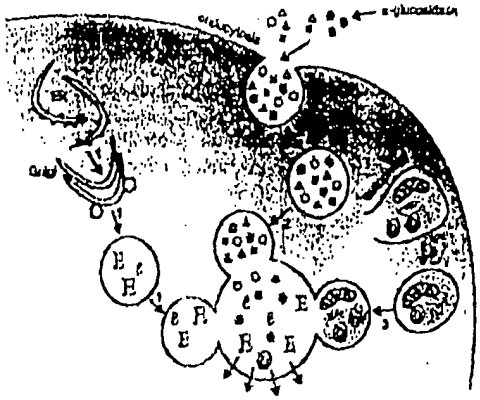

Fig. 1. Enzyme replacement therapy in lysosomal storage diseases. Newly synthesised lysosomal enzymes enter the lumen of the endoplasmic reticulum co-translationally. They are glycosylated, folded, and equipped with the M6P recognition marker as lysosomal targeting signal (route 1). Inside lysosomes, they catalyse the digestion of biological compounds that are delivered by endocytosis (extracellular) (route 2) and autophagy (intracellular) (route 3). Inherited deficiency of lysosomal enzymes leads to lysosomal storage diseases. Lysosomal enzymes administered to cells are taken up via bulk (inefficient) and receptor mediated (efficient) endocytosis and are delivered to the lysosomes where they can supplement the missing enzyme. (*E* enzymes, *ER* endoplasmic reticulum)

Advanced technology is required to secure substantial enzyme supplies over long periods. Around 1980, the focus shifted from enzyme therapy to bone marrow transplantation [15].

A few people pursued the original concept and gave enzyme therapy a second and better chance by applying newly acquired knowledge on receptor mediated endocytosis. Ashwell and Morell [2] were among the pioneers to demonstrate the role of the asialoglycoprotein receptor (galactose) in uptake of glycoproteins by hepatocytes. The mannose receptor was shown to facilitate uptake of proteins with mannose-terminating carbohydrate side chains by Kupffer cells and macrophages of spleen and bone marrow [29], and fibroblasts were found to exchange lysosomal proteins via the mannose 6-phosphate (M6P) receptor [14, 21]. Brady and his group [3] were the first to demonstrate the potential of receptor mediated enzyme therapy for lysosomal storage disease in a patient with Gaucher disease. The glucocerebrosidase used in the study was purified from human placental tissue. The complex carbohydrate side chains were trimmed by sequential action of neuraminidase, galactosidase and N-acetyl-glucosaminidase in order to expose mannose residues and thereby target the enzyme to Kupffer cells and macrophages in spleen and bone marrow; the major sites of lysosomal glycolipid storage in Gaucher disease. The study turned out successful enough to convince scientists, patients and industry of the feasibility of receptor mediated enzyme therapy for Gaucher disease [4].

Enzyme therapy for Pompe disease

We have worked along parallel lines to investigate the feasibility of receptor mediated enzyme therapy for Pompe disease. Table I lists the critical events by date.

Table I. Enzyme therapy for Pompe disease from science to industrial enterprise

Event	Production	
	Milk enzyme	CHO enzyme
Enzyme therapy: proof of principle	[35]	
AGLU cDNA cloning	[16]	
	[24]	
AGLU gene cloning	[17]	
	[25]	
Production	[6]	[11]
	[7]	[36]
Testing in animals	[7]	[36]
	[8]	[22]
		[7]
Industry enters the scene	Pharming (1991)	Synpac (1994)
	Genzyme (1998)	KDL BioTech (2000)
		Genzyme (2000)
		Pharming (2000)
		Novazyme (2000)
Orphan drug designation	1996	1997
Phase I trial	1998	-
Phase II trial	1999	-
Phase I/II trial	-	1999
Phase II trial data	[31]	[1]
Phase II/III trial	Not planned	May 2001

The target tissues in Pompe disease are muscle and heart. Patients have the same acid AGLU deficiency in all tissues, but the lysosomal glycogen accumulation and the symptomatology are largely restricted to skeletal muscle when the residual enzyme activity is 5%-25% of the normal range. At lower activity levels, heart and other tissues become increasingly involved. There is typically a clinical spectrum from early onset very severe to late onset mild disease. Affected infants have cardiomegaly around birth. They present as floppy babies and die usually before 1 year of age due to cardiorespiratory failure. Onset of symptoms is delayed, and cardiomegaly prevented by low levels of residual AGLU activity. In extreme cases, the skeletal muscle weakness may remain obscure until the sixth decade [27].

Our initial studies on the feasibility of enzyme replacement therapy for Pompe disease were directed towards establishing the presence of M6P receptors on cardiomyocytes and skeletal muscle cells and testing whether these could be employed to facilitate uptake of acid AGLU [26]. To this end, muscle biopsies of patients were dissociated with collagenase and trypsin, and myoblasts (satellite cells) were taken into culture. It turned out no problem to correct the lysosomal glycogen storage in these cells by addition of M6P-containing acid AGLU to the culture medium [32, 33, 34]. Herewith, the first requirements were fulfilled, but the experimental set-up does not mimic the reality in detail. When enzyme therapy is performed via intravenous infusion, the muscle cells do not come into direct contact with the enzyme like they do in tissue culture. The endothelial barrier of the capillaries needs to be crossed and the interstitial connective tissue (endomysium) needs to be passed. In this respect, the situation in Pompe disease is far different from that in Gaucher disease where there are no barriers between the Kupffer cells and the enzyme in the circulating blood. In Fabry disease the situation also is favourable compared to Pompe disease because the endothelial cells are a primary target. Nevertheless, we did obtain uptake of acid AGLU in muscle and heart of mice after intravenous administration of M6P-containing AGLU from bovine testis. The uptake of AGLU without M6P was less [35].

Technical challenges and financing

It took another 8 years before the first clinical trial of enzyme therapy in Pompe disease started. The time was spent on the development of technology to produce therapeutic grade recombinant human AGLU on a large scale. Therapy for Gaucher disease started with the heroic action of Genzyme Corporation (Boston, USA) to produce tailor-made glucocerebrosidase from tons of human placentas. The investment paid off for all parties. Patients with non-neurological forms of Gaucher disease (type 1) obtained an effective medicine [12]. Scientists were pleased because their ideas were realised. The company had shown its strength by bringing a new drug on the market and obtained returns.

The Orphan Drug Legislation, lending certain attractive rights to companies marketing medicines for rare diseases, has played an important role in this development. The past 5 years have shown concerted action of scientists, patients, industry and investors to be a golden formula for developing enzyme therapy for lysosomal storage diseases. Belief in enzyme therapy has returned. More importantly, the first reports confirming efficacy have appeared and clinical trials are ongoing for at least four of the lysosomal storage diseases [1, 10, 20, 28, 31]. In all instances, the protocols are directed to receptor-mediated tissue targeting, and recombinant DNA technology is applied for controlled large-scale enzyme production. The investment climate is excellent. The market seems profitable enough to have several companies competing for the same product. Both TKT (Boston, USA) and Genzyme Corporation have launched enzyme therapy for Fabry disease. Four companies are presently engaged in the development of enzyme therapy for Pompe disease. Genzyme/Pharming/Synpac as conglomerate and Novazyme (Oklahoma City, USA) as newcomer on the lysosomal disease market. As of August 2001, Genzyme and Novazyme have merged.

Back in 1991 there was no company strong and experienced enough to take up the challenge of producing recombinant acid AGLU for the treatment of Pompe disease, but research continued. Two Chinese hamster ovary (CHO) cell lines expressing high levels of recombinant enzyme were produced in university centres using the same AGLU cDNA but different vector systems [11, 36]. Using our CHO cell line, we produced a sufficient amount of enzyme to deliver proof of the principle of enzyme therapy in a mouse model of Pompe disease [7]. Simultaneously, therapeutic effect was shown in Japanese quails with the disease using the other CHO cell line [22]. Meanwhile, a completely different technology emerged which involved the production of medicines in the milk of transgenic animals [18]. Genomic DNA constructs are typically used in this production process in contrast to cDNA constructs employed in CHO cells (Fig. 2). The acid *AGLU* gene is linked to the promoter region of the bovine α_{S1}-casein gene that promotes high level expression in epithelial cells of the mammary gland. The construct is introduced in the animals' genome by injection into the pronucleus of fertilised oocytes. Embryos are implanted in foster mothers and a line of transgenic animals is obtained by germline transmission. The enzyme is harvested from the milk. Both production systems require downstream processing. The recombinant human acid AGLU from either milk or CHO cell media needs to undergo several rounds of purification before it can be administered intravenously to patients. The end products from CHO cells and milk are very similar in molecular mass (110 kD), and kinetic properties [7, 11, 36]. The carbohydrate composition may vary slightly depending on the enzyme source.

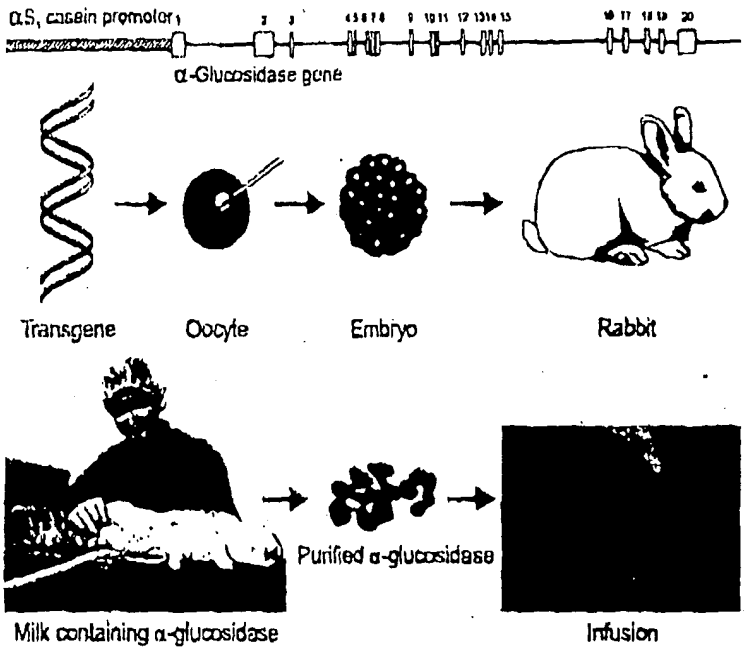

Fig. 2. Recombinant human AGLU from rabbit milk. The human acid *AGLU* gene is fused to 6.3 kb of the bovine α_{S1}-casein promoter and as such injected in the pronucleus of fertilised rabbit oocytes to generate transgenic founders. A transgenic line is obtained by breeding. Females produce recombinant human enzyme in the mammary gland during lactation and secrete the product in their milk. The enzyme is extracted from the milk in several purification steps and administered intravenously to the patients

Recombinant acid AGLU from CHO cells and mouse milk, both produced in our own laboratory, are equally efficiently taken up by cultured fibroblasts of patients with Pompe disease via the M6P receptor. However, the same enzyme from rabbit milk is taken up less efficiently by cultured fibroblasts. Interestingly, the uptake of the two enzymes by the target tissues of mice is not consistently different (unpublished results). More extensive studies are needed to verify these initial findings.

Clinical studies

Recombinant human acid AGLU from both sources were finally tested in the clinic (Table 1). Dr Chen of Duke University, North Carolina, USA reported at the SIIS symposium in Fulda the results of his study with CHO enzyme. We have summarised below the design and outcome of our study in which the enzyme from rabbit milk was used [31].

Study design and outcome

The aim of the study was to test safety and efficacy of recombinant human AGLU from rabbit milk in patients with the severe infantile form of Pompe disease. For inclusion, patients had to have the combination of generalised muscle weakness, cardiomegaly, acid AGLU deficiency and glycogen storage in skeletal muscle. Patients older than 10 months and those who were dependent on artificial ventilation were excluded. Four patients were included: two with an advanced stage of disease (7 and 8 months old), and two younger patients who were in significantly better condition at inclusion (2.5 and 3 months old). The two older patients were practically immobile at the time of inclusion. They required supplemental oxygen and had signs of cardiac instability. One of them became respirator dependent directly after inclusion before the start of treatment. The other patient became ventilator dependent after 10 weeks of treatment during a bout of pneumonia. One of the younger patients had signs of cardiac decompensation and respiratory distress at birth and was fed by nasogastric tube. The fourth patient was diagnosed at birth when he showed cardiomegaly on a chest X-ray film that was taken for disease unrelated indication. Both younger patients manifested axial hypotonia, head lag and slipping through.

The recombinant human AGLU from rabbit milk was administered intravenously in a weekly dose of 15–20 mg/kg (at start of treatment) to 40 mg/kg (at present) and is generally well tolerated. Transient reactions are seen sometimes during infusion, such as fever, malaise, erythematous rash, sweating, flushing and tachycardia. All are manageable without medication. After 12 weeks of treatment with the low dose, the acid AGLU activity in muscle had increased from 1%–2% of normal (before treatment) to 12%–28%, the levels typically measured in late-onset Pompe disease. During the 12 following weeks with high dose, the activity increased to normal levels. After 36 weeks of treatment, we observed improvement in muscle morphology in the younger patient who was in the best condition at the start of treatment. Muscle tissue sections stained less intense for glycogen (PAS staining) and muscle fibres appeared less damaged. Similarly clear

changes were at that time not yet evident in muscle biopsies of the other three patients.

Cardiac changes were monitored by ultrasound. The left ventricular mass index of all patients, exceeding approximately three times normal at inclusion, decreased after start of treatment (up to 25% of baseline for one of the patients). Signs of cardiac instability disappeared in all cases.

All patients gained strength over the first 36 weeks of treatment. They learned to play with toys. The two younger patients perform better than the two older ones. One of the younger patients learned to lift her legs from the surface and touch her feet whilst playing. At present she can sit independently. The other, with the best condition at start of treatment, has learned to sit and crawl at 9.5 months of age. At 12 months he could creep and stand with support of one arm and he could walk at 16 months. Importantly, all patients have well passed the age of 1 year which is more than the average life expectancy of patients with infantile Pompe disease. All four patients receive continuous treatment to evaluate the long-term effect of enzyme replacement therapy on motor and meutal development and overall quality of life.

Results of two trials

Comparing the studies with recombinant human AGLU from rabbit milk and CHO cells, there are differences and similarities in outcome. One patient in each study responds very well. The patient treated with rabbit milk enzyme had a characteristic cardiomegaly at birth. The patient treated with CHO enzyme had a normal baseline cardiac evaluation with a left ventricular mass at two standard deviations above the norm (close to the P98) when treatment was started at 3 months after birth. Obviously, we are confronted with clinical diversity preventing in part the comparison of data. Further, dose level and infusion frequencies were different in the two trials. A second patient in the study with rabbit milk enzyme responds well in that she has acquired the ability to roll over and sit and has remained ventilator independent over the first 72 weeks. The patient is homozygote for the deltaT525 mutation and does not produce endogenous acid AGLU. Her progress demonstrates that a cross-reactive immunological material (CRIM) negative status is not a priori inhibitory for successful treatment. In contrast, the two CRIM negative patients in the trial with CHO enzyme were said to respond initially well to the treatment, but their condition declined when high antibody titres developed against the recombinant enzyme. The other patients in both trials are CRIM positive to some extent. The difference in antibody response is multi-interpretable. First of all, the enzyme preparations used are probably not identical with respect to precise molecular structure, notably the carbohydrate composition. Second, the two preparations may contain various types and degrees of impurities and are formulated differently. This may affect their immunogenicity. Moreover, the dosing regimen was different at the two test sites. All these factors, separate or together, may explain why CRIM negative patients respond differently to the two enzyme preparations.

Prospects and challenges

The positive effects of enzyme therapy for Pompe disease are too strong to deny. For the benefit of patients, enzyme therapy ought to be brought to the market. It requires a Phase III trial to deliver final proof of therapeutic effect. The current pilot studies with rabbit milk and CHO enzyme indicate that quick and convincing results can be obtained by extension of studies in patients with the infantile form of Pompe disease; but, at the same time, it seems inevitable that not all included patients will respond equally well. The problem can in part be managed by careful definition of inclusion and exclusion criteria and proper dosing. A second challenge is to prove efficacy of enzyme therapy in late onset Pompe disease. The milder affected patients live longer and are continuously confronted with loss of quality of life. It is essential for them to implement therapy at the earliest possible moment in order to prevent irreversible damage of muscle function. In theory, it is easier to correct the enzyme deficiency in late onset than in early onset disease because the residual AGLU activity is significantly higher in the former than in the latter condition. On the other hand, it has to be awaited whether adult muscle tissue is equally accessible for the enzyme and equally reparable as growing muscle of infants.

As it stands, the prospects of enzyme therapy for Pompe disease are good, but hurdles still need to be overcome. In April 2000, Genzyme-Pharming LLC announced the discontinued development of enzyme replacement therapy with recombinant human AGLU from rabbit milk. The companies stated that they believed production in CHO cells to be quicker. A new study with enzyme from CHO cells was started in May 2001. All together, that is more than 35 years after the first trials were undertaken. Patients, investigators and companies are eagerly awaiting the outcome.

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